antiarrhythmic therapies. Accompanying dysfunctional Ca^{2+} handling, increased mitochondrial reactive oxygen species (ROS) production is also observed in the diseased heart. Since mitochondria are in close proximity to the redox-sensitive sarcoplasmic reticulum (SR) Ca^{2+} release channels (ryanodine receptors, RyRs) and the SR Ca^{2+} ATPase (SERCA), mitochondria-derived ROS could play a crucial role in modulating Ca^{2+} cycling under pathological conditions. Previous work has shown that mitochondrial depolarization and ROS-induced ROS release significantly enhance spontaneous SR Ca^{2+} release (Ca^{2+} spark frequency) in resting myocytes, but the role of mitochondria-derived ROS on Ca^{2+} dynamics and action potentials in paced cardiac myocytes has not been examined. We hypothesize that the pathological mitochondrial ROS burst forms a ROS microdomain between mitochondria and SR, altering the proximal SR Ca^{2+} handling channels and consequently leading to disturbed Ca^{2+} cycling and abnormal electrical activity. To test this hypothesis, we developed a new multiscale myocyte model that incorporates mitochondria and local Ca^{2+} control, and links RyRs and SERCA to the ROS environment surrounding the SR. The simulations show that the mitochondria-derived ROS burst stimulates RyRs and inhibits SERCA, inducing a cytosolic Ca^{2+} ($[Ca^{2+}]$i) transient. This extra $[Ca^{2+}]$i transient activates the Na+/Ca^{2+} exchanger and Ca^{2+}-sensitive nonspecific cationic channels, forming a transient inward current (Iti) that evokes early or delayed afterdepolarizations. This study defines the role of mitochondria-derived ROS in Ca^{2+} overload-mediated ventricular arrhythmias, and underscores the importance of considering mitochondrial targets in designing new antiarrhythmic drugs in the context of sudden cardiac death.

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Mitochondrial Ca^{2+} Dynamics in the Heart

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Compelling reports suggest that during an intracellular $[Ca^{2+}]_i$ transient a large influx of Ca^{2+} enters the mitochondrial matrix and that this influx is followed by an equally enormous Ca^{2+} efflux. These Ca^{2+} fluxes underlie a dramatic increase and then decrease in matrix free Ca^{2+}, $[Ca^{2+}]_{mito}$ Yet, other equally compelling investigations suggest that small fluxes occur with little or no measureable changes. Here we take advantage of the high temporal and spatial resolution of confocal microscopy imaging to examine this question in primary cultures of rat and rabbit ventricular cardiomyocytes (48 hours). Using a mitochondrially targeted Ca^{2+}-sensitive fluorescent protein ''Mitycam'', we examined the time course of the changes in $[Ca^{2+}]_{mito}$ in isolated ventricular myocytes.

Additionally, we have examined isolated Mitycam-containing mitochondria to investigate the rapidity of the kinetics of the changes in $[Ca^{2+}]_{mito}$ as measured by Mitycam in response to changes in extra-mitochondrial $[Ca^{2+}]$ (0 to 100uM). The measured changes in $[Ca^{2+}]_{mito}$ occurred within milliseconds. We therefore conclude that Mitycam (which co-localizes with mitochondrial-specific markers such as MitoTracker red), is not rate-limiting when it reports changes in $[Ca^{2+}]_{mito}$ of ~1 s) in intact (i.e. non-permeabilized) cardiac ventricular myocytes where a large change in cytosolic $[Ca^{2+}]_i$ (from ~100 nM to ~10 uM) following caffeine (10 mM) application. However, in these same cells, physiologic $[Ca^{2+}]_i$ transients produced no significant measureable increase in $[Ca^{2+}]_{mito}$ in a beat-to-beat manner. They do, however, report modest time-averaged changes in $[Ca^{2+}]_{mito}$ following changes in heart rate. These observations suggest that $[Ca^{2+}]_{mito}$ responds to changes in $[Ca^{2+}]_i$ like a low-pass-filter.

We conclude that the cardiac $[Ca^{2+}]_i$ transient does not significantly change $[Ca^{2+}]_{mito}$ in cardiac myocytes in a beat-to-beat manner but slowly influences the time-averaged $[Ca^{2+}]_{mito}$ signal.

3116-Pos Board B271

Mitochondrial Calcium Uptake: Context Matters

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Mitochondrial calcium (Ca^{2+}) fluxes regulate ATP generation, contribute to the regulation of apoptosis, and when very large can play a role in buffering changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). However, fundamental disagreements on the extent and speed of Ca^{2+} uptake by mitochondria have been reported. The reliability and consistency mitochondrial Ca^{2+} uptake measurements is consequently critical for our understanding of cell biology and pathology in cells from all tissue including heart, neurons, and kidney. Importantly, measurement of Ca^{2+} movement across the inner mitochondrial membrane (IMM) has been dramatically enhanced by the molecular identification of the mitochondrial Ca^{2+} uniporter (MCU) and the mitochondrial Na^+/Ca^{2+} exchanger (NCLX). First, we quantitatively analyze cardiac mitochondrial Ca^{2+} uptake experiments from the literature. We then interpret the results with respect to measurements conducted on recent MCU candidates which suggest that the conductance of a single MCU is ~6-7 pS (in 105 mM $[Ca^{2+}]$). Our quantitative analysis suggests three clear findings: 1. Under physiological conditions, Ca^{2+} influx into mitochondria is small and is dwarfed by other cytosolic Ca^{2+} extrusion pathways; 2. MCU-dependent Ca^{2+} uptake appears to be dependent on $[Ca^{2+}]_i$ under physiological conditions; 3. There appear to be hundreds of MCU channels per mitochondrion but they are predominantly closed under physiological conditions. We conclude that under physiological conditions mitochondria do not act as a significant Ca^{2+} buffers in heart despite being capable of substantial Ca^{2+} accumulation if non-physiological conditions favor prolonged levels of high extra-mitochondrial $[Ca^{2+}]$.

3117-Pos Board B272

Lamotrigine Increases Intracellular Calcium Levels and Camkii Activation in Mouse Dorsal Root Ganglion Neurons

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Lamotrigine is a neuroprotective agent that is used clinically for the treatment of seizures and neuropathic pain. A significant volume of literature has reported that lamotrigine exerts analgesic effect by blocking Ca^{2+} channels. However, little is known regarding the effect of lamotrigine on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The aim of this study was to determine whether lamotrigine modulates $[Ca^{2+}]_i$ in sensory neurons. Lamotrigine-induced changes in $[Ca^{2+}]_i$ were measured in mouse dorsal root ganglia (DRG) neurons using the Ca^{2+}-sensitive fluorescent indicator Fluo 3-AM and a confocal laser scanning microscope. Ca^{2+}/calmodulin-dependent kinase II (CaMKII) activation was assessed by fluorescence intensity using immunocytochemical procedures. Treatment with 1, 10, 30, or 100 μM lamotrigine transiently increased $[Ca^{2+}]_i$ in DRG neurons in a dose-dependent manner. Treatment with 100 μM lamotrigine induced a significant (three-fold) increase in the Ca^{2+} peak in the presence or absence of extracellular Ca^{2+}. The lamotrigine-induced Ca^{2+} increase was abolished or decreased by treatment with a specific PLC inhibitor (U73122), IP3R antagonist (xestospongin C), or RyR antagonist (dantrolene). In some cells, treatment with 100 μM lamotrigine caused a transient Ca^{2+} increase, and the Ca^{2+} levels quickly fell to below the basal Ca^{2+} level observed prior to lamotrigine application. The decrease in basal Ca^{2+} levels was blocked by treatment with a CaMKII inhibitor (KN93). Immunocytochemical analysis indicated that lamotrigine treatment increased the expression of phosphorylated CaMKII in DRG neurons.

Treatment with lamotrigine increased $[Ca^{2+}]_i$ apparently as a result of Ca^{2+} release from intracellular stores and CaMKII activity.

Key words: Calcium, Ca^{2+}/calmodulin-dependent kinase II, Dorsal Root Ganglia, Lamotrigine

3118-Pos Board B273

Calcium Buffering in the Nerve Terminals of the Posterior Pituitary

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Nerve terminals possess a variety of mechanisms to regulate Ca^{2+} signaling, and these mechanisms in turn provide a variety of avenues for regulating exocytosis and neurosecretion. Endogenous Ca^{2+} buffers are important Ca^{2+} regulating molecules that can have an especially profound influence on the spatiotemporal dynamics of intracellular Ca^{2+} signals. These proteins rapidly bind free Ca^{2+} within several hundred nanometers of open Ca^{2+} channels, limiting the rise of free Ca^{2+} in the nerve terminal and restricting its spatial spread. Ca^{2+} sequestration and extrusion mechanisms subsequently act to restore resting levels of free Ca^{2+}. By modulating Ca^{2+}, these Ca^{2+} buffering, sequestration and extrusion mechanisms play an important role in regulation Ca^{2+} dependent exocytosis. In the present work, intracellular Ca^{2+} signaling in the peptidergic nerve terminals of the rat posterior pituitary was investigated using two-photon fluorescence imaging with the Ca^{2+}-sensitive fluorescent dye fluo-8. Ca^{2+} imaging together with simultaneous measurement of Ca^{2+} current by patch clamp recording provided a direct assessment of the cytoplasmic Ca^{2+} buffering capacity. These experiments showed that the cytoplasmic Ca^{2+} buffering capacity declined as cytosolic $[Ca^{2+}]$ rose, and thus indicated that the Ca^{2+} dye and endogenous buffers saturated in the range of $[Ca^{2+}]$ studied. These data were interpreted using a model for the $[Ca^{2+}]$ dependence of buffering capacity that takes binding site saturation into account. This analysis yielded values for K_d